METHOD AND APPARATUS FOR GENERATING THERMAL MELTING CURVES IN A MICROFLUIDIC DEVICE

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] Pursuant to 35 U.S.C. §§119 and/or 120, and any other applicable statute or rule, this application is a continuation-in-part of U.S. Ser. No. 11/032,749, filed Jan. 11, 2005, which is a continuation of U.S. Ser. No. 10/003,472, filed on Nov. 15, 2001, which claims the benefit and priority of U.S. Ser. No. 60/249,578, filed on Nov. 16, 2000. The disclosures of all of those applications are incorporated herein by reference.

TECHNICAL FIELD

[0002] The present invention relates to the characterization of biological materials on a microfluidic device. More particularly, embodiments of the present invention are directed toward determining the thermal properties of biological materials on a microfluidic device.

BACKGROUND OF THE INVENTION

[0003] When carrying out chemical or biochemical analyses, assays, syntheses or preparations, a large number of separate manipulations are performed on the material or component to be assayed, including measuring, aliquotting, transferring, diluting, mixing, separating, detecting, incubating, etc. Microfluidic technology miniaturizes these manipulations and integrates them so that they can be executed within one or a few microfluidic devices.

[0004] For example, pioneering microfluidic methods of performing biological assays in microfluidic systems have been developed, such as those described by Parce et al. in U.S. Pat. No. 5,942,443 entitled "High Throughput Screening Assay Systems in Microscale Fluidic Devices", and Knapp et al. in PCT Publication No. WO 98/45481 entitled "Closed Loop Biochemical Analyzers". Additionally, microfluidic devices for performing temperature-mediated reactions have been explored by Stern in U.S. Pat. No. 6,670,153.

[0005] One type of biological assay of particular interest in many fields of science is the detection and quantification of binding between various molecules. For example, screening of numerous compounds or molecules to determine how they bind to one another or how they bind to a particular target molecule is extremely important in many areas of research. For example, screening of large libraries of molecules is often utilized in pharmaceutical research. "Combinatorial" libraries, composed of a collection of generated compounds, can be screened against a particular receptor to test for the presence of possible ligands and to quantify the binding of any possible ligands.

[0006] Various methods exist to characterize the binding between molecules. Many of those methods involve calorimetric analysis. Isothermal calorimetry (ITC) and differential scanning calorimetry (DSC) are examples of such methods. By measuring the thermal parameters of a binding reaction, calorimetry can be used to test for the presence of binding between the molecules by detecting a shift in the thermal denaturation of a molecule that occurs when another molecule is bound to it. The shift in the thermal denaturation of a molecule (which could be as expressed in a molecular melt curve) can be monitored via the fluorescence of an indicator dye that binds to only select conformational states of the molecule. Alternatively, in some cases the binding between molecules can be determined by changes in the intrinsic fluorescence of one of the molecules.

[0007] Characterization of the binding between molecules is also important tool in the characterization of nucleic acids. For example, Knapp et al. in U.S. Published Application No. 2002/0197630 entitled "Systems for High Throughput Genetic Analysis" discuss the use of melting curve analysis to detect single nucleotide polymorphisms (SNPs). Molecular melt curves (and differences between molecular melt curves) can also be used to detect and analyze sequence differences between nucleic acids. The thermal denaturation curve for nucleic acids can be monitored by, e.g., measuring thermal parameters, fluorescence of indicator dyes/molecules, fluorescence polarization, dielectric properties, or the like.

[0008] A welcome addition to the art would be a process that allows rapid binding assays to be performed on a microfluidic device with minimal use of compounds and reagents. The current invention describes and provides these and other features by providing methods and microfluidic devices for performing binding assays using molecular melt curves. These and other features of the invention will be made clear upon review of the following.

SUMMARY OF THE INVENTION

[0009] The present invention provides methods, systems, kits and devices for conducting binding assays using molecular melt curves in microfluidic devices. Molecule(s) to be assayed can be flowed through microchannels in the devices where the molecule(s) optionally are exposed to additional molecules constituting, e.g., fluorescence indicator molecules and/or binding partners of the molecule being assayed. The molecules involved are then heated (and/or cooled) and a detectable property of the molecules is measured over a range of temperatures. From the resulting data, a thermal property curve(s) is constructed which allows determination and quantification of the binding affinity of the molecules involved.

[0010] In one aspect, methods of generating a thermal property curve for at least one molecule in a microfluidic device are provided. The methods comprise flowing the molecule(s) into a microchannel, heating the molecule(s) in the microchannel, detecting at least one detectable property of the molecule(s) during the heating, and, generating a thermal property curve for the molecule(s) from such data. The methods provided involve observation of changes in at least one physical property of the one or more molecule(s), e.g., fluorescence, which results from, e.g., unfolding or denaturing, or from altering of one or more additional physical property of the molecule, in response to changes in temperature and as a result of binding.

[0011] The methods are applicable to numerous types of molecular interactions, including those of proteins, enzymes, nucleic acids (either double-stranded or single-stranded), ligands, peptide nucleic acids, cofactors, receptors, substrates, antibodies, antigens, polypeptides, etc., with one or more additional molecule or moiety. The methods of